Long-Term Studies of the Red Imported Fire Ant, *Solenopsis invicta*, Infected with the Microsporidia *Vairimorpha invictae* and *Thelohania solenopsae* in Argentina

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ABSTRACT A study was conducted on populations of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), infected with the microsporidia *Vairimorpha invictae* Jouvenaz and Ellis (Microsporidia: Burenellidae) and *Thelohania solenopsae* Knell, Allen, and Hazard (Microsporidia: Thelohaniidae). Fire ant populations and microsporidia prevalence were monitored three to five times per year for 3–4 yr in eight field plots in northern Argentina. The mean population index per plot showed an overall reduction of 69%. The percentage of infection with *V. invictae* and *T. solenopsae* showed fluctuations that ranged from 29.2 to 1.4% and 13.6 to 2.6%, respectively. The highest infection rates were observed at the beginning of the study. A total of 394 colonies were sampled during the study: 325 (82.5%) were healthy and 69 (17.5%) were infected with microsporidia. The proportion of infected colonies with brood was 81% (56/69), similar to the proportion of healthy colonies with brood (78%; 255/325). The proportion of infected and healthy colonies in the population index categories was significantly different. Of the infected colonies with brood, 49.3% were medium and 1.4% were large in size. In contrast, healthy colonies were generally larger, with 29.7 and 10.4% being medium and large, respectively. The general environmental conditions in the area of the plots were appropriate for fire ant population growth; consequently, they do not explain the overall reduction in the populations. These results, combined with additional evidence reported previously, suggest that infection with *V. invictae* and *T. solenopsae* has a deleterious effect on native populations of *S. invicta*.

KEY WORDS *Solenopsis invicta*, *Vairimorpha invictae*, *Thelohania solenopsae*, population index, biological control

AFTER THE ACCIDENTAL INTRODUCTION of the red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel (Hymenoptera: Formicidae), respectively, into the United States >80 yr ago, they have become aggressive pests of people, domestic animals, agriculture, and wildlife, first in the southeastern United States and Puerto Rico (Adams and Lofgren 1981) and more recently in several locations in New Mexico and California. A classical biological control approach for fire ants has been considered since the 1970s (Allen and Buren 1974, Williams and Whitcomb 1974, Jouvenaz 1983, 1990, Wojcik 1986. Wojcik et al. 1987) and has been emphasized since 1988 with an intensive search of effective biological control agents by the USDA-ARS-SABCL, mostly in Argentina, but also in neighboring countries. The pathogens *Vairimorpha invictae* Jouvenaz and Ellis (1986) (Microsporidia: Burenellidae) and *Thelohania solenopsae* Knell, Allen, and Hazard (1977) (Microsporidia: Thelohaniidae) are obligate intracellular microorganisms specific to fire ants (Briano et al. 2002). They were originally discovered in *S. invicta* in Mato Grosso, Brazil, and later found in other species of South American fire ants such as *S. richteri* Forel, *S. quinquecuspis* Forel, and *S. macdonaghi* Santschi (Jouvenaz et al. 1980, Wojcik et al. 1987, Briano et al. 1995d, 2002, Briano and Williams 2002). The first long-term field studies were conducted on one of the recovered organisms, *T. solenopsae,* and the black imported fire ant, *S. richteri,* host (Briano et al. 1995a). After finding *T. solenopsae* in the U.S. fire ant populations (Williams et al. 1998), this microsporidium has been part of field trials for biological control of imported fire ants. Oi and Williams (2002) and Oi et al. (2004) studied the prevalence and effect of *T. solenopsae* on field colonies of *S. invicta* in the United States. The microsporidium *V. invictae* is another important component of the complex of natural enemies that attack fire ants in South America. Its natural occurrence and effect on the host under laboratory conditions were recently reported (Briano and Williams 2002); however, the

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Table 1. Summary of sampling in plots in Santa Fe, Argentina, May 2000 to March 2004

Plots	Number of colonies (%)						Population index change (%)
	Total	Healthy	Infected				
			V. invictae	*T. solenopsae*	Dual	Total	
1	34	28 (82.4)	6 (17.6)	0	0	6 (17.6)	−65
2	91	68 (74.7)	11 (12.1)	10 (11.0)	2 (2.2)	23 (25.3)	−60
3	76	66 (86.8)	7 (9.2)	2 (2.6)	1 (1.3)	10 (13.2)	−53
4	70	67 (95.7)	1 (1.4)	2 (2.9)	0	3 (4.3)	−100
5	44	34 (77.3)	4 (9.1)	6 (13.6)	0	10 (22.7)	−78
6	24	14 (58.3)	7 (29.2)	3 (12.5)	0	10 (41.7)	−63
7	37	32 (86.5)	3 (8.1)	2 (5.4)	0	5 (13.5)	−56
8	18	16 (88.9)	0	2 (11.1)	0	2 (11.1)	+213
Total	394	325 (82.5)	39 (9.9)	27 (6.9)	3 (0.8)	69 (17.5)	−69

effect of these infections on red imported fire ant populations in their native land was never reported. Thus, the objective of this study was to document the long-term effects of both microsporidian pathogens on field populations of the red imported fire ant.

Materials and Methods

After a survey conducted in north central Argentina in 1999 by Briano and Williams (2002), it was determined that the northeastern Santa Fe province was the most appropriate area for a long-term study of red imported fire ant populations that are infected with microsporidia. The colonies of *S. invicta* in this area have shown the highest infection prevalence. *V. invictae* reached epizootic levels at times. Six field plots were established in May 2000 between the localities of Reconquista and San Justo, on the roadsides of Route 11 at the following kilometer markers: plot 1, km 760 (29°17′57.7″ S; 59°52′04.1″ W); plot 2, km 750 (29°21′03.9″ S; 59°57′05.3″ W); plot 3, km 707.6 (29°35′34.4″ S; 60°13′59.0″ W); plot 4, km 677.5 (29°51′38.3″ S; 60°16′58.2″ W); plot 5, km 624.8 (30°19′05.5″ S; 60°23′30.5″ W); and plot #6, km 600 (30°31′44.4″ S; 60°27′21.9″ W). A variable percentage of fire ant colonies were initially infected with the microsporidia *V. invictae* and/or *T. solenopsae* except those in plot 4 (control). Two extra control plots free of diseases were established in August 2001, also along the roadsides of Route 11 near the locality of Perez Cello at the following kilometer markers: plot 7, km 662 (29°59′38.4″ S; 60°18′28.8″ W) and plot 8, km 659 (30°01′14.1″ S; 60°18′46.8″ W). All plots were rectangular in shape, 10 by 100 m (0.1 ha) and were monitored three to five times per year until March 2004. For each plot, the monitoring consisted of (1) the number of active colonies, (2) the evaluation of fire ant populations using the USDA population index (PI) method (Harlan et al. 1981, modified by Lofgren and Williams 1982), and (3) the microscopic examination of the worker samples from each colony for the presence of microsporidian spores. According to gas chromatography studies performed at the USDA-ARS-Center for Medical, Agricultural and Veterinary Entomology at Gainesville, FL, the fire ant species present in the plots was mainly *S. invicta.* Occasionally, a few colonies of *S. macdonaghi* were found.

Each colony was assigned a PI of 1, 2, 3, 4, or 5 for colonies without worker brood or of 5, 10, 15, 20, or 25 for colonies with worker brood, relative to a visual estimation of the number of ants per colony (<100 to >50,000; see categories in Table 2). A PI for each plot and sampling date was obtained by summing the PIs assigned to the colonies in each plot. The percentage change in the PI per plot (dPI) was obtained as follows: dPI = $(y-x)/x \cdot 100$ or $\frac{y-x}{x}100$; where x and y are the initial and final PI, respectively.

Worker ants were collected by introducing a talc-dusted 7-ml vial into the mounds for a few seconds to several minutes according to the air temperature and activity of the ants. The vial acted as a pitfall trap in the surface of the mounds. The workers trapped (n = 300–5,000) were preserved in 70% ethanol for transportation to the laboratory, where they were macerated and examined under a phase-contrast microscope (400×) for the presence of *V. invictae* and *T. solenopsae* spores. This procedure was successfully demonstrated by Jouvenaz et al. (1977). The presence of brood was confirmed in each colony by visual observation of immature forms after disturbing the ant nests minimally.

Rainfall records were obtained for the localities of San Justo and Vera, located near the plots at Route 11 km 567 and km 721, respectively. Additional information collected at the plots on every sampling date included air temperature; vegetation height (arbitrarily ranked as short, <5 cm; medium, 5–15 cm; or tall, >15 cm); soil condition (arbitrarily ranked as dry [hard and difficult to excavate], moist [soft and easy to excavate, no excess of water], or wet [muddy]); general weather condition at the moment of the collection (arbitrarily ranked as bad [overcast, cold, windy, and/or rainy], good [partially cloudy, cool, moderate wind], or excellent [warm, sunny, no wind); and use of the land where the plots were located (arbitrarily classified as crops or natural fields). The presence in the plots of other ant genera and other known fire ant natural enemies was recorded.

The χ^2 test was used to compare the proportion of infected and healthy colonies with and without worker brood and the proportion of infected and

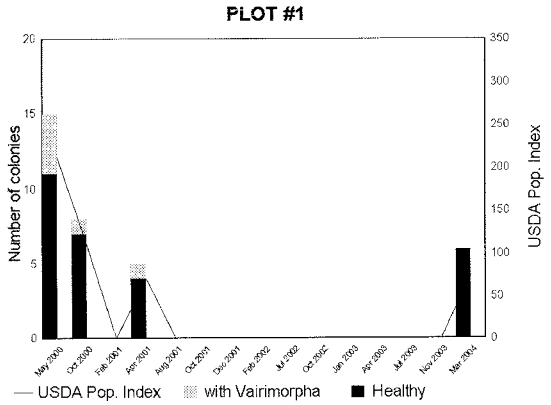

Fig. 1. Monitoring of microsporidian infections in *S. invicta*, plot 1, Santa Fe, Argentina.

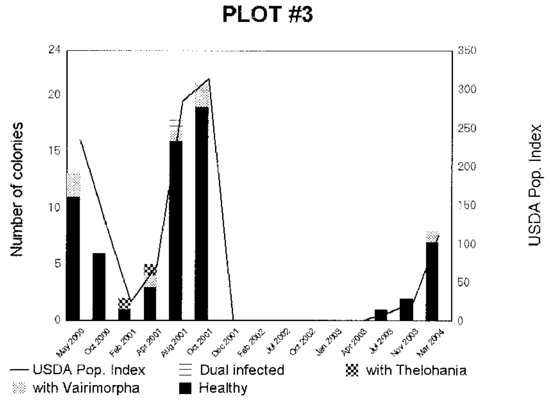

Fig. 3. Monitoring of microsporidian infections in *S. invicta*, plot 3, Santa Fe, Argentina.

healthy colonies in each PI category. A linear regression model was used to fit the trend of the mean PI per plot and the trend of the mean percentage of infection per plot. Because of sample size limitations (lack or few colonies in some categories) and because no differences were observed in the percentage of colonies with and without brood, categories 1–2 and 4–5 were grouped with 5–10 and 20–25, respectively, for statistical analysis.

Minitab Statistical Software (1991) was used to analyze the statistical tests.

Results and Discussion

The original design of this observational study with five infected plots and three control plots (since August 2001) was impossible to maintain until the end of the study because control plots 4, 7, and 8 became infected with microsporidia 18–23 mo after the plots were established. This was an impediment both in the development of the study and the analysis of the results. Natural infections of control field plots are common in areas where these diseases are present and were reported in other studies (Briano et al. 1995a, Oi and Williams 2002). The results are presented for each of the individual plots.

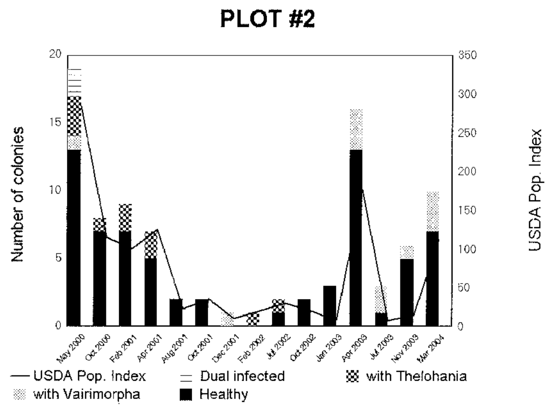

Fig. 2. Monitoring of microsporidian infections in *S. invicta*, plot 2, Santa Fe, Argentina.

Plot 1. The initial fire ant density was 15 colonies, 4 (26.7%) of which were infected with *V. invictae* (Fig. 1). This was the only plot that was infected solely with *V. invictae* during the entire study period. The total number of colonies sampled, including those that became established during the course of the study, was 34. Six (17.6%) were infected (Table 1). An abrupt decrease in colony density with 100% reduction in the number of active colonies was observed in February 2001. A moderate reinfestation was observed, and a second 100% reduction was observed in August 2001. After that, the plot was free of fire ants for 31 mo. At the end of the study in March 2004, a reinfestation of six healthy colonies was observed. In total, a 65% reduction of fire ants was observed.

Plot 2. The initial fire ant density was 19 colonies, with 6 (31.6%) infected with *V. invictae* and/or *T. solenopsae* (Fig. 2). This was one of two plots in which dual infections appeared in the same fire ant colony (one colony). The total number of colonies sampled over the course of the study was 91, 23 (25.3%) of which were infected (Table 1). The percentage of infected colonies ranged from 13 to 100%. Decreases in colony density followed by high and moderate reinfestations were observed in April 2003 and March 2004. The plot was never free of fire ants, although the density was very low (one to three colonies per plot) for 17 mo. At the end of the study, the fire ant population was 60% lower than at the initiation.

Plot 3. The initial fire ant density was 13 colonies, and 2 (15.4%) were infected with *V. invictae* (Fig. 3). This was the second plot in which dual infections appeared in one fire ant colony. The total number of colonies sampled during the study was 76, 10 (13.2%) of which were infected with *V. invictae* and/or *T. solenopsae* (Table 1). The percentage of infection ranged from 11 to 50% of the colonies. As in plot 2, a continuous decrease in colony density was observed from the beginning of the study until February 2001,

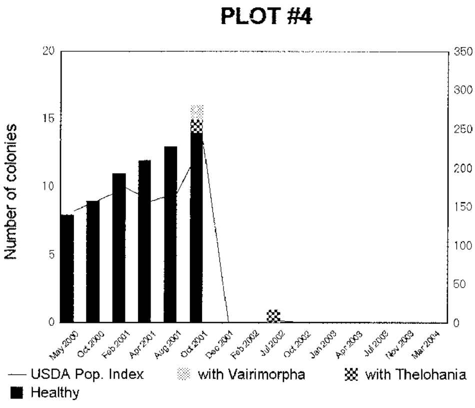

Fig. 4. Monitoring of microsporidian infections in *S. invicta*, plot 4, Santa Fe, Argentina.

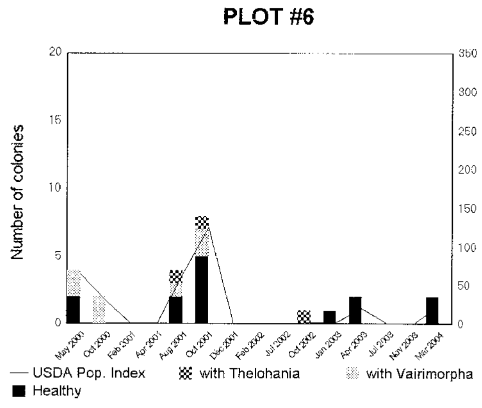

Fig. 6. Monitoring of microsporidian infections in *S. invicta*, plot 6, Santa Fe, Argentina.

followed by a strong reinfestation from April to October 2001. A 100% reduction in the number of active colonies was verified in December 2001, and the plot was free of fire ants for 19 mo. A moderate reinfestation was observed at the end of the study in July 2003. Most of these new colonies were healthy. The ant population was 53% lower at the end of the study compared with the beginning.

Plot 4. This plot was originally a control plot. The initial fire ant density was eight disease-free colonies (Fig. 4). A gradual increase in colony density was observed during the first 17 mo of the study, until October 2001, when two colonies were found infected: one with *V. invictae* and the other with *T. solenopsae.* By that date, the increase in the PI was almost 62%. A subsequent abrupt decrease in colony density was observed in December 2001, with a 100% reduction in the number of active colonies. The plot was nearly free of fire ants until the end of the study (27 mo). The total number of colonies sampled was 70, and 3 (4.3%) were infected (Table 1). After microsporidian infections occurred, the total number of colonies found was only 17, and the percentage of infected colonies was 17.6%. Between 13 and 100% of the colonies were infected during the study period. No colonies were present at the termination of the experiment.

Plot 5. The initial fire ant density was five colonies; two (40%) were infected with *T. solenopsae* (Fig. 5). The total number of colonies sampled was 44, 10 (22.7%) of which were infected with *V. invictae* and/or *T. solenopsae* (Table 1). The percentage of infected colonies ranged from 14 to 100%. As in plots 2 and 3, decreases in colony density followed by light or high reinfestations were observed. The plot was free of fire ants for two short periods of 3 and 6 mo. A total population reduction of 78% was observed.

Plot 6. The initial fire ant density was four colonies, two (50%) of which were infected with *V. invictae* (Fig. 6). The total number of colonies sampled was 24, with 10 (41.7%) infected with *V. invictae* and/or *T. solenopsae* (Table 1). The percentage of infection ranged from 38 to 100% of the colonies. Similarly to plots 2, 3, and 5, decreases in colony density were observed followed by light reinfestations. The plot was free of fire ants for several periods of 6–20 mo. A reduction of 63% of fire ants was observed.

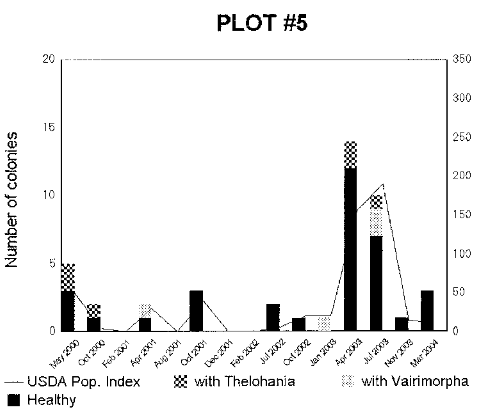

Fig. 5. Monitoring of microsporidian infections in *S. invicta*, plot 5, Santa Fe, Argentina.

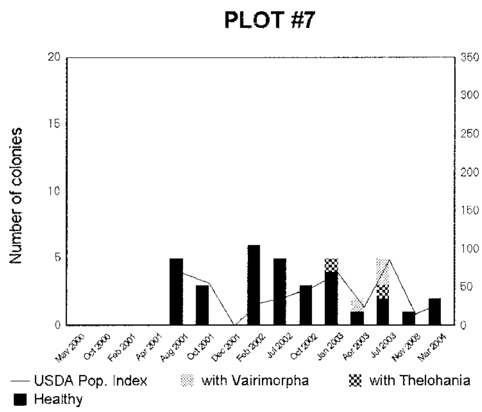

Fig. 7. Monitoring of microsporidian infections in *S. invicta*, plot 7, Santa Fe, Argentina.

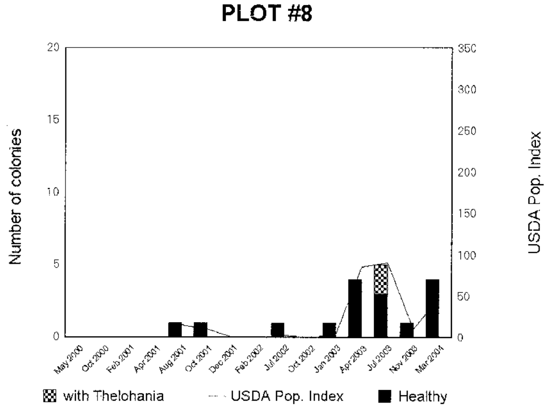

Fig. 8. Monitoring of microsporidian infections in *S. invicta*, plot 8, Santa Fe, Argentina.

Plot 7. This plot was originally a control plot. The initial (August 2001) fire ant density was five disease-free colonies (Fig. 7). The fire ant density was stable during the study except in December 2001, when no colonies were found. In January 2003, colonies in the plot were infected with *T. solenopsae*, and soon after, with *V. invictae.* The total number of colonies sampled was 37, 5 (13.5%) of which were infected (Table 1). In fact, after colonies in the plot developed microsporidian infections, the total number of colonies declined to 15, and the percentage of infected colonies increased to 33.3% (range, 20–60%). A final reduction of 56% of the fire ant population was observed.

Plot 8. This plot was originally a control plot. The initial fire ant density was only one disease-free colony in August 2001 (Fig. 8). In April 2003, an increase in colony density represented a 560% increase in the total ant population. The plot was observed to be infected with *T. solenopsae* in July 2003, the only plot infected only with this microsporidium throughout the study. The total number of colonies sampled was 18, 2 (11.1%) of which were infected (Table 1). After *T. solenopsae* was first observed, the total number of colonies found was 10, and the percentage of infection was 20%. A final population increase of 213% was observed.

The mean **PI** per plot over the entire study showed fluctuations with abrupt reductions and moderate reinfestations (Fig. 9). An important average overall population reduction of 69% was observed; the PI decreased from 165.3 in May 2000 to 51.4 in March 2004 (Fig. 9; y = 100.83 − 6.19x; r^2 = 0.36). This is consistent with the long-term study conducted previously with populations of *S. richteri* infected with *T. solenopsae* in Buenos Aires Province, where a reduction of 83% of the number of active colonies was reported (Briano et al. 1995a). Although fluctuations in fire ant populations of *S. invicta* infected with *T. solenopsae* in the United States are common (Oi and Williams 2002), such marked reductions have not been reported (S. Porter, personal communication). However, the drastic reduction in the density of fire ants observed in December 2001 in most of the plots (plots 3, 4, 5, 6, and 7) was a sampling limitation rather than a collapse of the populations and was the result of hot and dry soil conditions that made the workers move deeper within the nests, and consequently, difficult to sample. The dry condition of the soil was temporary and was not the result of long droughts (Fig. 12).

For the complete study period (Table 1), a total of 394 colonies was sampled in all plots; 325 (82.5%) were healthy, and 69 (17.5%) were infected: 39 colonies

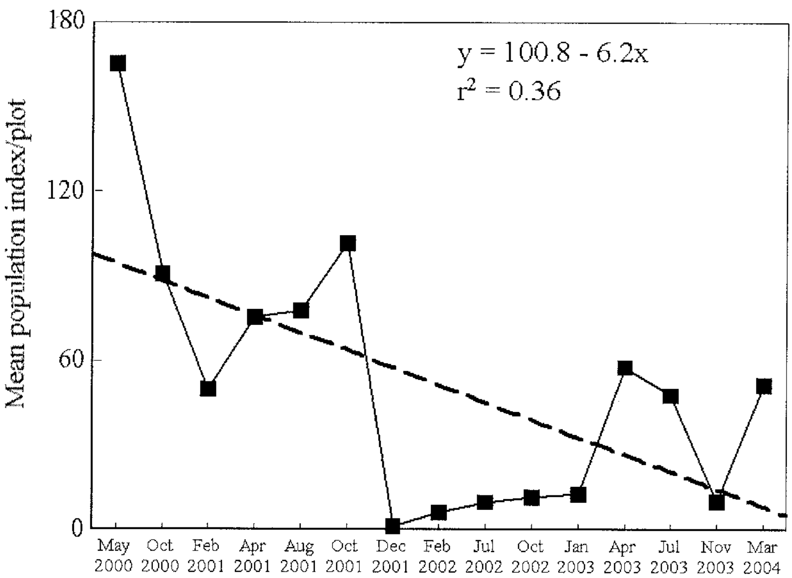

Fig. 9. Mean PI per plot, May 2000 to March 2004, Santa Fe, Argentina.

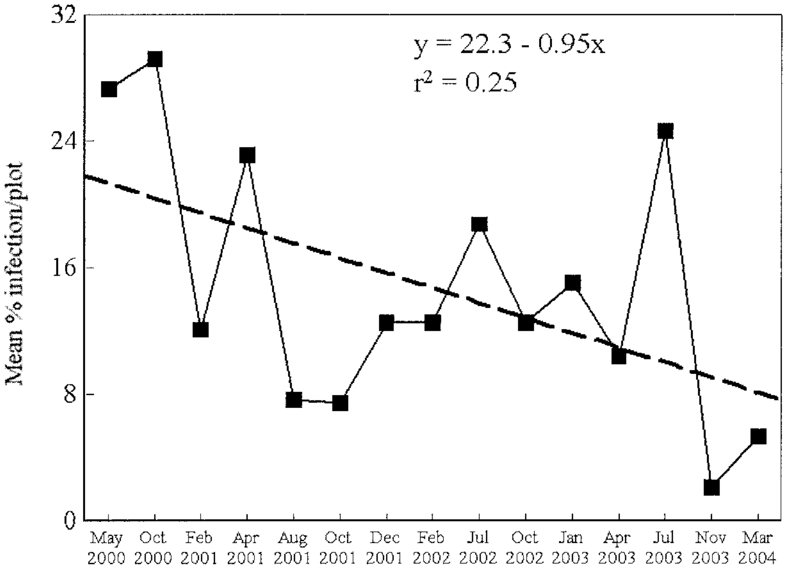

Fig. 10. Mean percentage of infection per plot, May 2000 to March 2004, Santa Fe, Argentina.

(9.9%) were infected with *V. invictae* in seven of the eight plots, 27 (6.9%) were infected with *T. solenopsae*, also in seven plots, and only 3 colonies (0.8%) were infected with both microsporidian species in only two of the plots.

The overall percentage of infection with *V. invictae* and *T. solenopsae* also showed important fluctuations that ranged from 29.2 to 1.4% and 13.6 to 2.6%, respectively. The highest infection rates (>26%) were observed at the beginning of the study, whereas the lower ones (<5%) were observed at the end (Fig. 10; $y = 22.3 - 0.95x$; $r^2 = 0.25$). This may indicate that these pathogens act in a density-dependent manner and infect more ant colonies as the density of fire ant colonies increases (Tanada and Kaya 1993).

Briano et al. (1995d) and Briano and Williams (2002) reported a lower prevalence of *V. invictae* (2.3%), a similar prevalence for *T. solenopsae* (8%), and a much lower prevalence of dual infections (0.2%). However, the percentage of field colonies with dual infections reported here (0.8%) was similar to the combined probability of finding *V. invictae* (9.9%) and *T. solenopsae* (6.9%) infecting simultaneously the same colony (0.099 by 0.069 = 0.0068 = 0.7%). This probability prediction is consistent with previous findings (Briano and Williams 2002). Colonies infected with both pathogens at the same time (dual infections) were not found in two consecutive examinations. Contrary to the report for *S. richteri* (Briano and Williams 2002), where dual infections did not seem to exert extra stress on the colonies, the low persistence of dual infections in the field might indicate that they exert a highly detrimental synergistic effect for the entire colony. However, if colonies with dual infections collapse before transmitting the infections to other healthy colonies, the detrimental effect would be limited to individual colonies. This needs further and urgent investigation with laboratory tests designed specifically to evaluate the effect of dual infections.

The proportion of infected colonies containing worker brood was 81% (56/69) and was similar to the proportion of healthy colonies containing worker brood (78%; 255/325). The proportion of infected colonies without worker brood was 19% (13/69), similar to the proportion of healthy colonies without worker brood (22%; 71/325; $\chi^2 = 0.294$; df = 1; $P > 0.5$).

The proportion of infected and healthy colonies in the PI categories was significantly different for medium and large colonies with worker brood (catego-

Table 2. Proportion of infected and healthy colonies in the population index categories

Category	Colonies without worker brood					Colonies with worker brood					Total
	1	2	3	4	5	5	10	15	20	25	
No. workers/colony	<100	101–1,000	1,001–10,000	10,001–50,000	>50,000	<100	101–1,000	1,001–10,000	10,001–50,000	>50,000	
No. infected colonies (%)	0	6 (8.7)	6 (8.7)	0	1 (1.4)	0	7 (10.1)	34 (49.3)[a]	14 (20.3)	1 (1.4)[a]	69 (100)
No. healthy colonies (%)	1 (0.3)	24 (7.3)	36 (11.0)	9 (2.7)	1 (0.3)	3 (0.9)	46 (14.1)	97 (29.7)[a]	75 (22.7)	34 (10.4)[a]	325 (100)

[a] $\chi^2 = 7.174$; df = 2; $P < 0.05$.

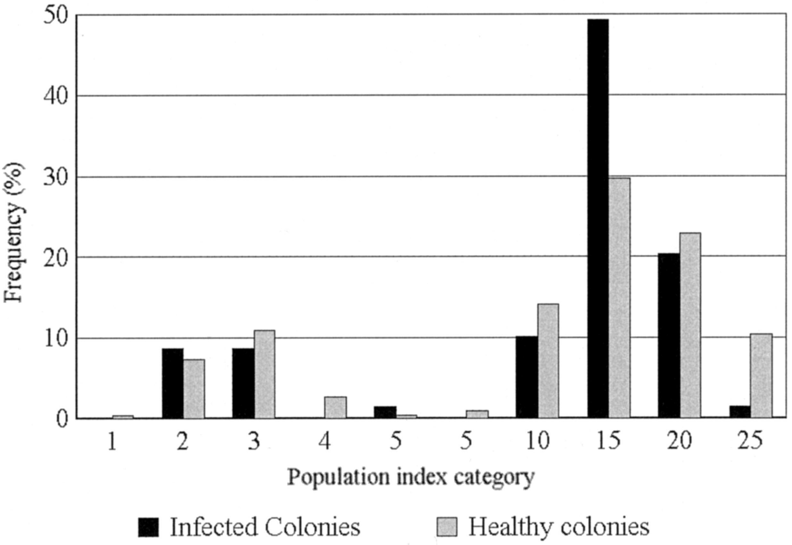

Fig. 11. Frequency distribution (%) of fire ant colonies in the categories of the PI, Santa Fe, Argentina.

ries 15 and 25; Table 2; Fig. 11). For infected colonies, the proportion of medium and large colonies was 49.3 and 1.4%, respectively, and for healthy colonies, it was 29.7 and 10.4%, respectively ($\chi^2 = 7.174$; df = 2; $P < 0.05$). Of the 35 large colonies, 34 (97.1%) with worker brood (category 25) were healthy, indicating that infected fire ants did not produce large colonies. It is also possible that large colonies became reduced in size as they became infected. However, no direct evidence was obtained in this regard, because fire ants move their nests very often (Briano et al. 1995c), and individual colonies were not followed through the study. The effect observed in mound size is consistent with Briano et al. (1995b), who reported similar results for colonies of *S. richteri* infected with *T. solenopsae* in Argentina, and with Cook (2002) and Oi and Williams (2002), who reported smaller colony sizes of *S. invicta* infected with *T. solenopsae* in the United States.

The general environmental conditions in the area of the plots during the study period were suitable for fire ant population growth: mild temperatures, abundant rain, short vegetation, and suitable soil condition. The

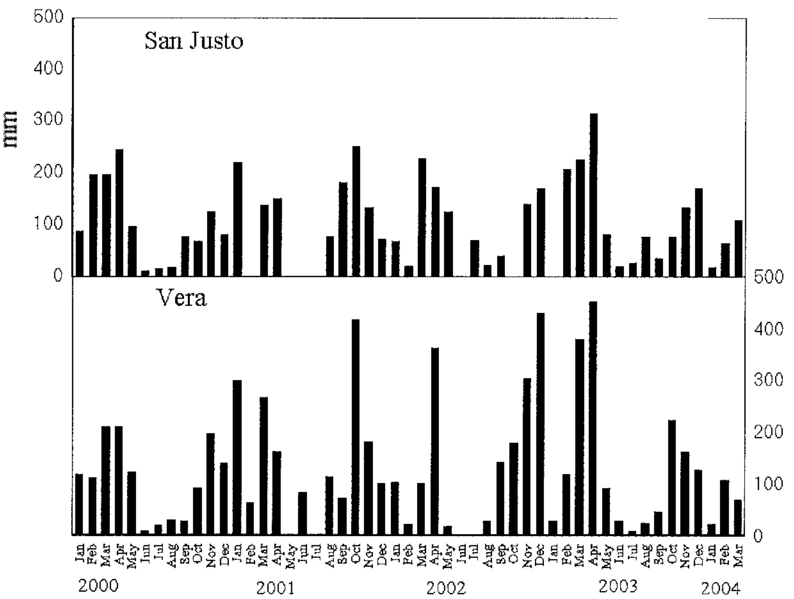

Fig. 12. Rainfall records (mm) for two localities near the plots, Santa Fe, Argentina, from January 2000 to March 2004.

mean air temperature at the plots at the moment of collections ranged from 15.4°C in July 2003 to 30.6°C in February 2002. Rainfall during the study period was abundant, with low peaks observed in the cold seasons and high peaks in spring and summer (Fig. 12). A short drought was observed in the San Justo area in May, June, and July 2001. Total annual rainfall ranged from 1,051 to 1,757 mm. Vegetation was short in 65% of the study periods, medium in 34%, and tall in only 1%. The soil condition was moist in 56% of the occasions, dry in 25%, and wet in 19%. The general weather conditions at the moment of the collections were excellent in 46% of the occasions, good in 43%, and bad (overcast, cold, windy) in 11%. The use of the land on which the plots were established consisted of 65% natural fields and 35% crops. A few nests of *Acromyrmex* sp., *Pheidole* sp., and *Crematogaster* sp. were occasionally found in or near the plots. Also, on some occasions, a few phorid flies (Diptera: Phoridae: *Pseudacteon* spp.) were seen in disturbed mounds.

In summary, although the loss of control plots obscured the conclusions of this study, local populations of *S. invicta* infected with *V. invictae* and *T. solenopsae* showed a substantial decrease in seven of the eight plots. In the original control plot (4), a gradual increase in the density of colonies was observed until the plot became infected. The overall reduction of the fire ant population based on mean PI per plot was 69% (range, 53–100%). Because climatic conditions were favorable for fire ant population increases, reductions observed in population densities must be associated with biotic factors. In five plots (plots 1, 3, 4, 5, and 6), there were long periods (19–34 mo) without fire ants, and in the remaining plots, the density of fire ants was usually very low. Nearly all large colonies were healthy. These results, combined with additional evidence reported previously such as the high degree of intracolonial prevalence of the two microsporidian species and high virulence in stressed workers and colonies under laboratory conditions (Briano et al. 1996, Briano and Williams 1997, 2002, Williams et al. 1999), suggest that the infection with *V. invictae* and *T. solenopsae* has deleterious effects on native populations of *S. invicta.* Additional studies should be conducted under quarantine conditions in the United States, especially with *V. invictae,* to allow its future introduction for the biological control of the red imported fire ants.

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